Quantification of Viable Bioaerosol Emanation from an ACMV System and Its Impact on Indoor Bioaerosol Pollution

Jin Wen Xiong[1], Man Pun Wan[2*], Bing Feng Ng[2], Siming You[3]

[1] Energy Research Institute @ NTU, Nanyang Technological University, 50 Nanyang Drive, 637553, Singapore

[2] School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore

[3] School of Engineering, University of Glasgow, James Watt South Building, G12 8QQ, Glasgow

Abstract

Viable bioaerosols can deposit and multiply in air-conditioning and mechanical ventilation (ACMV) systems. These bioaerosols can eventually enter indoor environments following the operation of the ACMV systems, contributing to indoor bioaerosol pollution. A method to identify and quantify viable bioaerosol emanation from an ACMV system and its impact on indoor bioaerosol pollution is proposed in this study. The method involves surface and air sampling of viable bioaerosols with analysis using a material-balance model. The proposed method was adopted to investigate the contribution of viable bioaerosol emanation from an ACMV system to indoor bioaerosol pollution in an air-conditioned room located in Singapore. The bioaerosol emanation rates from the ACMV system were found to be 2.4 CFU s^{-1} for viable bacteria and 3.9 CFU s^{-1} for viable fungi. Among various bioaerosol sources, bioaerosol emanation from the ACMV system was the largest source of indoor viable bacteria and the second largest source of indoor viable fungi in the air, behind the introduction of outdoor fungi through the mechanical ventilation. Potentially pathogenic bioaerosol species were also found in the ACMV system, which are in the genera of *Staphylococcus*, *Moraxella* and *Aspergillus*. Particularly, *Moraxella osloensis*, which is most likely to be emanated from occupants, was found to accumulate in the ACMV system, indicating the potential impact of the ACMV system's hygiene on indoor bioaerosol pollution. This study could contribute to the understanding of bioaerosol emanation from ACMV systems and their impact on indoor bioaerosol pollution. The proposed method could be used as a tool for analysing the potential sources of indoor bioaerosols and support the development of effective control measures against bioaerosol emanation from ACMV systems.

Keywords: Indoor bioaerosol dynamics; Natural ventilation; Bacteria; Fungi

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* Corresponding author. Tel: (+65) 67906957; Fax: (+65) 67906957

E-*mail address*: mpwan@ntu.edu.sg

NOMENCLATURE

A	Floor area (m^2)
C	Bioaerosol concentration (CFU m^{-3})
C_i	Indoor bioaerosol concentration (CFU m^{-3})
$C_{i,1}$	Contribution of initial bioaerosol concentration (CFU m^{-3})
$C_{i,2}$	Contribution of bioaerosol resuspension from the floor (CFU m^{-3})
$C_{i,3}$	Contribution of the potential bioaerosol emanation sources including occupants, the fresh air duct and the AC unit and the introduction of outdoor bioaerosols through the mechanical ventilation (CFU m^{-3})
E_0	The rate of supply of bioaerosols from outdoors through mechanical ventilation (CFU s^{-1})
E_1	Bioaerosol emanation rate of occupants (CFU s^{-1})
E_2	Bioaerosol emanation rate of the AC unit (CFU s^{-1})
E_3	Bioaerosol emanation rate of the fresh air duct (CFU s^{-1})
F	Sampling air flow rate of the impactor (14.15 L min^{-1})
N	Bacterial or fungal loadings on surfaces (CFU m^{-2})
N_{floor}	Bacterial and fungal loadings on the floor (CFU m^{-2})
n	Number of colonies on an agar plate (CFU)
R_{floor}	Resuspension rate of bacteria and fungi from the floor (# s^{-1})
r	Ratio of the volume (100 μL) of liquid sample spread on an agar plate to the volume (10 mL) of eluted ultra-pure water from one swab
S_t	Template area of surface sampling (0.005 m^2)
t	Time (s)
t_s	Sampling time for each air sample (10 minutes)
Q_f	Fresh air flow rate (m^3 s^{-1})
Q_r	Return air flow rate (m^3 s^{-1})
V	Volume of indoor space (m^3)
V_d	Deposition velocity of indoor bioaerosols on the floor(m s^{-1})
η_1	Filtration efficiency of the return air filter inside the AC unit

INTRODUCTION

Air conditioning and mechanical ventilation (ACMV) systems are widely used to provide a comfortable indoor environment (Cheong and Lau, 2003), especially in the tropical region where air-conditioning is needed all year round (Aziz et al., 2017). In Singapore, ACMV systems are widely adopted in buildings (Chen and Chang, 2012). In many cases, an ACMV system also serves as an engineering means to control indoor air quality (Zhao and Wu, 2009; Chen et al., 2016). However, whether an ACMV system is helping to control indoor air quality or is worsening indoor air quality remains debatable. An increasing number of studies have shown that ACMV systems can also serve as sources of indoor aerosols (Batterman and Burge, 1995; Bluyssen et al., 2003). Studies indicated that cooling coils of an ACMV system could be a potential aerosol source via air-flow-induced particle resuspension from the surfaces of the cooling coils when they were contaminated by particulate matter (Siegel and Carey, 2001; Siegel, 2002). Zhou et al. (2011) also found that particle resuspension from highly contaminated air ducts led to as much indoor aerosol pollution as that caused by indoor human activities, such as cleaning and playing for 60 minutes.

Bioaerosol, especially airborne bacteria and fungi, is an important concern for indoor environments (Lal et al., 2017). Human exposure to airborne bacteria and fungi can lead to serious infectious diseases (Cole and Cook, 1998; Daniels et al., 2016; Fan et al., 2017; Kim et al., 2017) such as Legionnaires' disease (Berrington and Hawn, 2013), tuberculosis (TB) (Pedersen et al., 2016) and pneumonic plague (Hammamieh et al., 2016). In addition, some allergens such as spores and hyphae (Jutel et al., 2016), which can cause serious respiratory diseases (Hargreaves et al., 2003; Vesper et al., 2008; Beck et al., 2012 ; Du et al., 2018), are found in indoor bioaerosols. Indoor bioaerosols consist of viable and non-viable portions (Nazaroff, 2016). Although non-viable bioaerosols can cause allergies when occupants are exposed to them (Kauffman and van der Heide, 2003), they do not multiply in the environment. The viable bioaerosols can deposit, accumulate,

and subsequently multiply on the surfaces of various components of an ACMV system under favourable conditions, exacerbating the biological contamination of the ACMV system (Batterman and Burge, 1995; Siegel and Carey, 2001; Zhao and Wu, 2006; Lu et al., 2009; Bonetta et al., 2010; Chen and Chang, 2012; Schmidt et al., 2012; Wu et al., 2016). These findings indicate the potential of ACMV systems to be bioaerosol emanation sources. Jo and Lee (2008) observed that switching on an ACMV system could lead to an instantaneous increase in concentrations of indoor airborne viable bacteria and fungi. However, little study has been conducted to quantify the bioaerosol emanation rate from ACMV systems and identify the species of bioaerosols emanated from ACMV systems.

This work experimentally investigates the emanation of viable bacteria and fungi from an ACMV system and its contribution to indoor bioaerosol pollution in a tropical indoor environment, where the environmental conditions are generally favourable to growth of microorganisms (Chen and Chang, 2012). Both air and surface sampling methods are employed for viable bioaerosol quantification and species identification. The result of this study could contribute to the understanding of bioaerosol emanation from ACMV systems and their impact on indoor bioaerosol pollution. The proposed method could also be used as a tool for analysing the potential sources of indoor bioaerosols and support the development of effective control measures against bioaerosol emanation from ACMV systems.

METHODOLOGY

Experimental Site

Experiments were conducted in a tutorial room (6.30 m [L] × 5.80 m [W] × 2.80 m [H]) located at Nanyang Technological University (NTU), Singapore. A schematic diagram of the tutorial room is shown in Fig. 1. The ACMV system of the room consists of a ceiling-mounted cassette-type air-

88 conditioning (AC) unit (plfy-p63vam-e, MITSUBISHI ELECTRIC) and a fresh air duct. The AC
89 unit draws room return air through a grill that has a Minimum Efficiency Reporting Value
90 (MERV)-4 rating return air filter installed, which provides a minimum filtration efficiency of less
91 than 20% for particles of diameters from 0.3 to 10.0 µm according to ASHRAE Standard 52.2
92 (ASHARE, 2017). The AC unit also draws outdoor air via a 22.8-m-long fresh air duct. A fresh air
93 filter of MERV-8 rating (minimum filtration efficiency of less than 10% for particles of diameters
94 from 0.3 to 1.0 µm, and that of less than 70% for particles of diameters from 1.0 to 3.0 µm, and
95 that of 70% to 85% for particles of diameters from 3.0 to 10.0 µm according to ASHRAE Standard
96 52.2 [ASHARE, 2017]) is installed at about 3.2 m downstream of the fresh air intake. The supply
97 air flow rate of the AC unit is 1400 m^3 hr^{-1}, a constant air volume. When the tutorial room is
98 operated in ACMV mode, the indoor air temperature set point is 24°C and relative humidity (RH)
99 set point is 60%, which is set according to the recommendation from Singapore Standard 554 (SSC,
100 2016). The tutorial room can also be operated in natural ventilation mode. There is a sliding window
101 (2.5 m^2) on the wall. When the room is naturally ventilated, the window is open and the AC unit is
102 switched off.
103
104 ***Air Sampling of Bioaerosols***
105 In this study, culture-based air sampling method was used to measure concentrations of indoor
106 viable bacteria and fungi in the air. The culture-based air sampling method is widely adopted for
107 measuring indoor bioaerosol concentrations, e.g., Singapore Standard SS554 (Iyengar, 2014),
108 WHO indoor air quality guideline (Heseltine and Rosen, 2009), Occupational Safety and Health
109 Administration (OSHA) (Kim et al., 2017), the Health and Welfare Department in Canada (Kim et
110 al., 2017) and Indoor Air Quality Association (Kim et al., 2017). Many studies employed culture-
111 based air sampling method to quantify bacterial and fungal concentrations for validating

predictions of indoor bioaerosol concentrations by mathematical formulas (Scheff et al., 2000; Green et al., 2003; Hathway et al., 2011; King et al., 2013).

Air sampling was conducted using a 200-hole, one-stage impactor (SKC) loaded with a petri dish with culture medium. A sampling flow rate of 14.15 L min^{-1} was provided by a sampling pump. The median cut-point (D_{50}) of the impactor at the sampling flow rate is 0.6 μm. Tryptic soy agar with addition of cycloheximide was used as the culture medium for bacteria. Malt extract agar with addition of chloramphenicol was used as the culture medium for fungi. Sampled bacterial agar plates were incubated for 48 hours in 35°C whereas fungal agar plates were incubated for 72 hours in 27°C, before colony counting. The colony counts were subsequently corrected using the method suggested by Macher (1989). The bacterial and fungal concentration (C, colony forming unit [CFU] per cubic meters) is calculated as $C = 1000n / (F \cdot t_s)$, where n (CFU) is the corrected number of colonies on a sampled agar plate. F = 14.15 L min^{-1} is the sampling air flow rate of the impactor with an agar plate inside. t_s = 10 min is the sampling time for each sample. Measurement uncertainty of indoor bacterial and fungal concentrations using the impactor is 7.67 CFU m^{-3}. Preparation of agar plates, and incubation and colony counting of air-sampled agar plates are described in Supplemental Material. We understand that CFU is not a direct reflection of bioaerosol amount. However, due to the fact that the culture-based air sampling method was used in this study, CFU m^{-3} is the representation unit of bioaerosol concentrations with this method.

Air sampling of indoor viable bacteria and fungi was conducted from 9:25 am to 4:35 pm in a day. There was a 20-minute interval between two successive air samplings. Therefore, fifteen pairs of air samples (15 air samples for bacterial cultivation and 15 air samples for fungal cultivation) could be collected in a day.

Surface Sampling of Indoor Surfaces

Surface sampling was conducted to quantify loadings of viable bacteria and fungi on indoor surfaces. Surface sampling was performed using SKC surface swab kits (SKC). A swab kit includes a sterile foam swab and a 5 cm × 10 cm plastic template that defines the target sampling area. During a surface sampling, the target area defined by the template was swabbed thoroughly in a rolling mode using the sterile foam swab. After sampling, the foam swab was kept in the sterile storage tube that came with the kit and was then transported back to the laboratory for culture-based analysis. To extract the surface sample from the swab kit, 10 mL of ultra-pure water was added to each storage tube containing the sampled swab. The tubes were then vortex-shaken in a vortex mixer (SPD Scientific) for one minute. The liquid sample eluted from the swab was then transferred to a clean tube. Next, 100 μL of the liquid sample was extracted by a pipette (HIRSCHMANN) and distributed on an agar plate for cultivation. Five repeats of agar plates were made for each eluted liquid sample. Incubation and colony counting of surface samples shares the same method as that for air sampling. Bacterial and fungal loadings on the sampled surface (N) can be calculated as $N = n / (r \cdot S_t)$, where n is the number of colonies on an agar plate. $r = 0.01$ is the ratio of volume of extracted liquid sample (100 μL) that distributed on an agar plate to the volume (10 mL) of eluted ultra-pure water from one swab (volume of liquid sample in the storage tube after adding 10 mL ultra-pure water) and $S_t = 0.005$ m^2 is the area that the plastic template defines for surface sampling.

158 ***Experiment Design***

159 ***Indoor Bioaerosol Model***

160 Damped areas such as rotted food or damaged furniture was not found in the tutorial room.
161 Before the experiments, air leakages of the room, such as door gaps, were sealed by tapes to
162 minimize the penetration of outdoor bioaerosols to the indoor space through the leakages.
163 Contribution of the penetration of outdoor bioaerosols through the leakages to indoor bioaerosol
164 concentration was assumed negligible. As such, introduction of outdoor bioaerosols through the
165 mechanical ventilation, bioaerosols emanated from occupants and the ACMV system, as well as
166 bioaerosol resuspension from the floor were the bioaerosol sources indoors when the room was
167 operated in the ACMV mode. When the room was operated in the ACMV mode, two assumptions
168 were made for the indoor bioaerosol dynamics in this study: 1) in the experiment, indoor
169 bioaerosols were assumed to be well mixed in the tutorial room. In a previous study, indoor aerosols
170 were assumed to be well mixed in an indoor space of a dimension of 5 m (L) × 6 m (W) × 3 m (H)
171 when the air mixing rate was 6 h^{-1} and duration of air mixing was 8 hours in the indoor space (Zhou
172 et al., 2011). In this study, dimensions of the tutorial room (6.30 m [L] × 5.80 m [W] × 2.80 m [H])
173 and duration of air mixing in the experiment (7 hours) were similar to those in the previous study
174 (Zhou et al., 2011). Meanwhile, the air mixing rate of the tutorial room in the ACMV mode was
175 13.7 h^{-1} in this experiment, which was more than double of that of the indoor space in the previous
176 study (Zhou et al., 2011). Therefore, indoor bioaerosols, as a sub-set of aerosols, could be assumed
177 to be well mixed in the experiment in this study. 2) Coagulation could be assumed to be negligible
178 due to low concentrations of indoor bioaerosols (less than 10^5 to 10^8 m^{-3}) (Zhou et al., 2011), which
179 can be found in the measurement results of indoor bioaerosol concentrations in the next section.
180 Then the material-balance equation of indoor bioaerosols when the room was operated in the
181 ACMV mode can be given by

$$V\frac{dC_i}{dt} = -C_iQ_f - Q_r\eta_1C_i + R_{floor}N_{floor}A - V_dC_iA + \sum_{j=0}^{3} E_j \ . \quad (1)$$

182 The term on the left-hand side of Eq. (1) is the time variation of amount of indoor bioaerosols in
183 the room. On the right-hand side of Eq. (1), C_iQ_f is the rate of indoor bioaerosol exfiltration. $Q_r\eta_1C_i$
184 is the bioaerosol removal rate by the return air filter. $R_{floor}N_{floor}A$ is the resuspension rate of
185 bioaerosols from the floor. V_dC_iA is the bioaerosol deposition rate on the floor. $\sum_{j=0}^{3} E_j$ is the
186 summation of emission rates from various bioaerosol sources. The solution to the Eq. (1) has the
187 following form:
188

$$C_i(t) = C_{i,1}(t) + C_{i,2}(t) + C_{i,3}(t) \ , \quad (2)$$

189 where $C_{i,1}$ indicates contribution of initial indoor bioaerosol concentration; $C_{i,2}$ denotes
190 contribution of bioaerosol resuspension from the floor; $C_{i,3}$ is contribution of bioaerosol emanation
191 sources including occupants, the fresh air duct, the AC unit and introduction of bioaerosols from
192 outdoors through the mechanical ventilation. These items can be fully expressed as:
193

$$C_{i,1}(t) = C_i(0)e^{-\frac{Q_f+Q_r\eta_1+V_dA}{V}t} \ , \quad (2a)$$

$$C_{i,2}(t) = \frac{R_{floor}N_{floor}A}{Q_f+Q_r\eta_1+V_dA}\left(1 - e^{-\frac{Q_f+Q_r\eta_1+V_dA}{V}t}\right) \ , \quad (2b)$$

$$C_{i,3}(t) = \frac{\sum_{j=0}^{3} E_j}{Q_f+Q_r\eta_1+V_dA}\left(1 - e^{-\frac{Q_f+Q_r\eta_1+V_dA}{V}t}\right) \ . \quad (2c)$$

The initial condition used to solve Eq. (1) is $C_i = C_i(0)$ at $t = 0$, where $C_i(0)$ is the indoor bioaerosol concentration measured at the beginning of the experiment.

A non-linear least squares method was applied to estimate the bacterial and fungal emanation rates by using the mathematical formula expressed by Eq. (2) to fit experimental results of indoor bacterial and fungal concentration. In the above equations, C_i is the indoor bioaerosol concentration. E_0 is the rate of supply of bioaerosols from outdoors through the mechanical ventilation, E_1 is the bioaerosol emanation rate of human occupants, E_2 is the bioaerosol emanation rate of the AC unit, and E_3 is the bioaerosol emanation rate of the fresh air duct. The fresh air flow rate Q_f can be calculated according to measurement results of air exchange rates (AER) and the volume of the tutorial room (V). The return air flow rate Q_r was measured to be 0.37 m^3 s^{-1}. In the indoor environment of the room, the resuspension rate, the filtration efficiency of the MERV 4-rating return air filter and the deposition velocity of indoor bioaerosols can be estimated according to aerodynamic diameters of the indoor bioaerosols (Thatcher and Layton, 1995; Lai, 2002; Zhou et al., 2011). In this study, targeted indoor bioaerosols are indoor viable bacteria and fungi in the air, which are the viable portion of indoor airborne bacteria and fungi. Typical ranges of aerodynamic diameters of the indoor airborne bacteria and fungi are from 0.5 to 2.5 μm (Wiedinmyer et al., 2004; Wiedinmyer et al., 2009) and from 2 to 4 μm (Madsen et al., 2016), respectively. Due to the fact that the typical range of aerodynamic diameters of the indoor airborne bacteria and fungi are larger than 0.5 μm, their deposition are mostly attributed to gravitational settling on upward facing horizontal surfaces (Whyte and Derks, 2015). Therefore, their deposition on and resuspension from sidewalls and the ceiling of the room can be neglected and thus the deposition velocity on the floor V_d and the resuspension rate from the floor R_{floor} are used in the Eq. (1). In the experiment, both the deposition velocity V_d and the resuspension rate R_{floor} were assumed

217 to be constant with time due to the constant supply air flow rate and a constant indoor activity
218 (sitting) in the tutorial room (Thatcher and Layton, 1995; Zhou et al., 2011). In the material-balance
219 equation, R_{floor}, V_d and the filtration efficiency of the return air filter η_1 are estimated according to
220 the aerodynamic diameters of the indoor bacteria and fungi and summarized in

Table **1**. As shown in Table 1, both the resuspension rate R_{floor} and the deposition velocity V_d are much less than one, indicating their insignificant impact on the indoor bacterial and fungal concentrations in the period of air sampling. According to the measurement results of filtration efficiency of a MERV 4-rating filter in a previous study (Burroughs, 2005), which has the same filter rating as the return air filter installed in the ACMV system of the tutorial room, both the variation of filtration efficiency of particles within the size range of 0.5 to 2.5 μm and that within the size range of 2 to 4 μm are insignificant, as shown in Table 1. Therefore, the average resuspension rate R_{floor}, the deposition velocity of indoor bioaerosols V_d and the filtration efficiency of the return air filter η_1 in Table 1 were used in the Eq. (1). They were calculated for the indoor airborne bacteria and fungi respectively. A is the indoor floor area (6.30 m [L] × 5.80 m [W]). Bacterial or fungal loadings on the indoor floor surface N_{floor} were measured by surface sampling using the method described in previous subsection.

Experimental Scenarios

In Eq. (1), E_j ($j = 0, 1, 2, 3$) are unknowns. Four experimental scenarios (Scenario 1–4, as shown in Fig. 2) were designed to determine these unknowns. In each scenario, air sampling of indoor viable bacteria and fungi repeated for five successive weekdays. Therefore, 75 pairs of air samples were collected for each scenario. Subsequently, a non-linear least squares method was applied to estimate the bacterial and fungal emanation rates, E_j ($j = 0, 1, 2, 3$), by using Eq. (2) to fit experimental results of indoor bacterial and fungal concentrations in each scenario. Before the start of each scenario, the AER of the room was measured by the tracer gas decay method using CO_2 (99.99% purity, Air Liquide) as the tracer gas. The CO_2 concentration was measured by a CO_2 meter (CM-0212, CO2Meter) at an interval of 20 seconds. Surface sampling was conducted on the first and the last day of each scenario to quantify bacterial and fungal loadings on the indoor floor

surface for estimating the contribution of their resuspension from the floor to indoor bioaerosol pollution. When the surface sampling was conducted, nine targeted sampling areas, which were evenly distributed on the floor area of the indoor space, were sampled using nine different swab kits. Due to the fact that the deposition velocity of indoor airborne bacteria and fungi is several magnitudes less than one, as shown in Table 1, their surface loadings on the floor can be assumed to be independent of their indoor concentrations during the air sampling experiment. In addition, the surface loadings are assumed to be constant during the air sampling due to the constant indoor supply air flow rate and activities of occupants in the tutorial room.

In Scenario 1, there was no occupant in the room and a HEPA filter was inserted at connection between the fresh air duct and the AC unit to prevent the introduction of outdoor bioaerosols through the mechanical ventilation and the emanation of bioaerosols from the fresh air duct. Hence, besides the resuspension from the floor (a common source in all scenarios), the bioaerosol emanation from the AC unit was the only emission source in this scenario. When Eq. (2) was applied to Scenario 1, $E_0 = E_1 = E_3 = 0$. As a result, the bioaerosol emanation rate of the AC unit (E_2) became the only unknown. Using the solution expressed by Eq. (2) to fit the experimental data from Scenario 1 through the non-linear least squares method, the emanation rates of the AC unit (E_2) for bacteria and fungi could be found.

Scenario 2 was largely similar to Scenario 1 but with addition of two occupants. Prior to Scenario 2, there were no occupants in the experimental room for 11 days. Applying Eq. (2) to Scenario 2, and using the E_2 determined from Scenario 1, $E_0 = E_3 = 0$, the bioaerosol emission from the occupants (E_1) was the only unknown in the equation. Using a similar fitting method for the experimental results obtained in Scenario 2, E_1 for bacteria and fungi could be determined.

In Scenario 3, the HEPA filter was moved to the fresh air intake to prevent the introduction of outdoor bioaerosols through the mechanical ventilation. However, this allowed for the bioaerosols

emanated from the fresh air duct to enter the room. For Scenario 3, $E_0 = 0$ in Eq. (2c). E_1 and E_2 were already determined from Scenario 1 and 2. The only unknown, the bioaerosol emanation rate of the fresh air duct (E_3), could be determined by fitting the experimental results obtained in Scenario 3 with Eq. (2) using the non-linear least squares method. Consequently, the bioaerosol emanation rate from the fresh air duct could be calculated.

In Scenario 4, the HEPA filter was removed. This scenario bore the closest resemblance to the actual operating condition of the room under ACMV mode. After determining E_1, E_2 and E_3 from the previous scenarios, E_0 (the rate of supply of bioaerosols from outdoors through mechanical ventilation) became the only unknown parameter in Eq. (2c) for Scenario 4. Using a similar data fitting method based on Scenario 4's air sampling data, E_0 could be quantified.

Table 2 summarizes the sources/sinks of indoor bioaerosols in each scenario. The contribution to indoor bioaerosol levels by the occupants can be illustrated by comparing Scenarios 1 and 2. Comparing Scenarios 2 and 3 and between Scenarios 3 and 4 reveal the contribution by the fresh air duct and the introduction of outdoor bioaerosols through the mechanical ventilation, respectively.

Two additional scenarios (Scenarios 5 and 6, as shown in Fig. 2) were defined to study the impact of the AC unit on indoor bioaerosol levels. In Scenario 5, the AC unit was disconnected from the fresh air duct, i.e., fresh air was supplied directly to the room without going through the AC unit. A standing fan was used to maintain indoor air mixing and thermal comfort. The air flow rate of the fan was 0.35 $m^3 s^{-1}$, which was similar to the return air flow rate of the AC unit (0.37 $m^3 s^{-1}$). On this basis, the deposition velocity (V_d) and the resuspension rate (R_{floor}) of indoor bioaerosols in this scenario were assumed to be the same as those in the other scenarios.

The AC unit could impact indoor bioaerosol levels in two ways, bioaerosol emanation or removal by its return air filter. In Scenarios 1 to 4, the AC unit operated with the return air filter and

293 contributed to indoor bioaerosol levels through these two mechanisms. Comparing Scenario 5 with
294 Scenario 4 reveals the net effect of the AC unit on indoor bioaerosol levels. Scenario 6 was designed
295 to further investigate the breakdown of these two mechanisms. In Scenario 6, the conditions were
296 almost the same as those in Scenario 4 except that the return air filter in the AC unit was removed.
297 The difference in indoor bioaerosol concentrations between Scenario 5 and Scenario 6 will reflect
298 the effects of bioaerosol emanation from the AC unit without the filtration effect of the return air
299 filter.

300 In the last scenario, Scenario 7, the room was naturally ventilated (the window was opened and
301 the AC unit was switched off) with the same standing fan used in Scenario 5 to provide thermal
302 comfort. Scenario 7 represented the condition of the room under natural ventilation mode. The
303 comparison between Scenario 7 and Scenario 4 reveals the relative efficacy of controlling indoor
304 bioaerosol levels between the natural ventilation mode and the ACMV mode.

305 After air sampling experiment in a day, the indoor floor area of the tutorial room was mopped
306 with clean water by a researcher to simulate the daily cleaning of the floor in the tutorial room on
307 weekdays. From Scenario 1 to Scenario 4 and in Scenario 6, which were operated under air-
308 conditioning, the ACMV system was switched on at 9 am and off at 5 pm in a day. In these
309 scenarios, indoor temperature ranged from 24 to 25°C while indoor relative humidity (RH) ranged
310 from 64% to 68% during the experiment. In Scenario 5 and Scenario 7, which were not operated
311 under air-conditioning, indoor temperature ranged from 27 to 28°C while indoor RH was around
312 70% during the experiment. In Scenario 7, which was operated in the natural ventilation mode, the
313 window was opened at 9 am and closed at 5 pm in a day. Average indoor temperature and RH
314 during the experiment in each scenario are shown in Supplemental Material Table S1. The
315 experiments were conducted from June to September 2016, which was within the southwest

316 monsoon season in Singapore, to minimize the influence of climate change on the differences of
317 indoor bioaerosol concentrations between the scenarios.
318
319 ***Species Identification of Bioaerosols***
320 In order to identify the species of the viable bacteria and fungi emanated from the ACMV system,
321 air sampling was conducted in two settings. In the first setting (Figs. 3 [a]), an impactor (sampler)
322 was placed at the end of the fresh air duct and connected with a reducer to isokinetically collect the
323 bioaerosols emanated from the fresh air duct while a HEPA filter was installed at the fresh air
324 intake to prevent the introduction of outdoor bioaerosols through the mechanical ventilation. As a
325 result, only the bioaerosols emanated from the fresh air duct was collected by the sampler. In the
326 second setting (Figs. 3[b]), the AC unit was disconnected from the fresh air duct. A chamber was
327 tightly connected to the return air grill of the AC unit and tapes were used to seal the gaps between
328 the chamber and the return air grill. Pure nitrogen gas was introduced into the chamber to free the
329 return 'air' from bioaerosols. Subsequently, a sampler was used together with the reducer to
330 isokinetically collect the bioaerosols in the supply air, which only carried the bioaerosols emanated
331 from the AC unit.
332 Air sampling was conducted in batches of 12 samples. After finishing a batch, the samples were
333 sent for incubation using the same method as described in the Supplemental Material. The colonies
334 on the incubated samples were then analysed based on their morphological characteristics using
335 standard taxonomic keys (Fotedar et al., 1991; Xu et al., 2008). This process continued until no
336 colony of new morphological characteristics was observed in the latest batch. This was to ensure
337 that all possible species were included. These agar plates were sent for species identification by the
338 DNA sequencing method. To briefly summarize, gDNA of the colonies was first extracted after
339 incubation and PCR reaction was subsequently performed to obtain the PCR product. The PCR

product is the fungal internal transcribed spacer (ITS) and bacterial 16s rRNA, which were targeted for the DNA sequence analysis following the method described in (Luhung et al., 2017).

RESULTS AND DISCUSSION

Impacts of Bioaerosol Emanation from ACMV System on Indoor Concentration

Average indoor bioaerosol concentrations from the seven scenarios are shown in Fig. 4. The averages are taken from all the air samples collected in five days (75 air samples for bacterial cultivation and 75 samples for fungal cultivation) of air sampling experiment for each scenario. The average indoor bacterial concentration in Scenario 2 was about twice that of Scenario 1, while their difference in fungal concentration was statistically insignificant ($p > 0.05$). This suggests that the occupants contributed significantly to the airborne bacterial concentration but had a minor impact on the fungal concentration indoors.

The average bacterial and fungal concentrations in Scenario 3 were significantly ($p < 0.05$) higher than their counterparts in Scenario 2, which indicated the fresh air duct as a possible source of airborne bioaerosols. Surfaces of the fresh air duct were loaded with bacteria and fungi, which could have originated from the deposition or growth of deposited microorganisms on the duct surfaces (Batterman and Burge, 1995; Bluyssen et al., 2003). Subsequently, the microorganisms on surfaces of the fresh air duct could be resuspended to form bioaerosols and transported indoors (Zhou et al., 2011; You and Wan, 2014).

The difference in the average bacterial concentrations between Scenario 3 and 4 was statistically insignificant ($p > 0.05$). However, the average fungal concentration in Scenario 4 was almost triple that of Scenario 3. This suggested that the fresh air intake had minor impacts on the bacterial concentration but was a major (or even the dominant) contributor to the indoor fungal concentration. It is also worth noting that this significant introduction of fungi from outdoors

364 through the mechanical ventilation was observed even though there was a MERV-8 rating filter
365 installed in the fresh air duct.
366 The difference in the average bacterial concentration between Scenario 5 and Scenario 4 was
367 also statistically insignificant ($p > 0.05$). However, the fungal concentration in Scenario 5 was 47%
368 higher than that in Scenario 4. This indicated that the AC unit had a significant effect on removing
369 fungi but had a minor effect on removing bacteria. Both the average bacterial and fungal
370 concentrations in Scenario 6 were significantly higher than those in Scenario 5 ($p < 0.05$). This
371 indicated the net effect of bioaerosol emanation from the AC unit (without removal of bioaerosols
372 by the return air filter).
373 Scenario 4 (ACMV mode) had a significantly lower average fungal concentration ($p < 0.05$) than
374 Scenario 7 (natural ventilation mode). The average bacterial concentration in Scenario 4 was about
375 30% higher than that in Scenario 7. This indicated that the ACMV mode was effective in
376 controlling the fungal level but led to a higher bacterial level as compared to natural ventilation.
377 This was caused by the bioaerosol emanation from the ACMV system and will be further
378 investigated in subsequent sections. The measured AER in the natural ventilation mode was about
379 double of that in the ACMV mode, leading to a higher introduction of outdoor fungi through the
380 ventilation in the former.
381
382 ***Bioaerosol Emanation Rates***
383 The average bacterial loading on the floor of the seven scenarios was calculated to be 2.6×10^5
384 CFU m^{-2} by averaging all the measurement results of bacterial loadings from the seven scenarios
385 (126 surface samples in total) and the average fungal loading was calculated to be 1.8×10^4 CFU
386 m^{-2} using the same method. As shown in Supplemental Material Table S2, variation of both the
387 bacterial and fungal loadings on the floor of the seven scenarios were insignificant, which might

388 be due to the constant supply air flow rate, the constant indoor activity during the experiment and
389 the regular cleaning after every day's experiment. Therefore, the calculated average bacterial and
390 fungal loadings on the floor of the seven scenarios are used as their floor surface loading N_{floor} in
391 the Eq. (1) respectively. As an initial estimate, the rate of bioaerosol resuspension from the floor
392 ($R_{\text{floor}}N_{\text{floor}}A$) was calculated to be 0.024 CFU s^{-1} for bacteria and 0.003 CFU s^{-1} for fungi.
393 Using Eq. (2) to fit the experimental data from Scenario 1 through the non-linear least squares
394 method, the emanation rates of the AC unit (E_2) for bacteria and fungi were found to be 0.87 CFU
395 s^{-1} and 2.96 CFU s^{-1}, respectively. The experimental results of Scenario 1 and the fitted curve of
396 Eq. (2) are shown in Supplemental Material Fig. S1.
397 Using a similar fitting method for the experimental results obtained in Scenario 2 (Fig. S2,
398 Supplemental Material), E_1 for bacteria and fungi were determined to be 1.11 CFU s^{-1} and 0.07
399 CFU s^{-1}, respectively. Since there were two occupants in the current experimental setting, it could
400 be estimated that the contribution from each occupant to bacteria is at a rate of 0.56 CFU s^{-1} and
401 fungi at a rate of 0.035 CFU s^{-1}.
402 The Bioaerosol emanation rate of the fresh air duct (E_3), could be determined by fitting the
403 experimental results obtained in Scenario 3 (Fig. S3, Supplemental Material) with Eq. (2) using the
404 non-linear least squares method. Consequently, the emanation rate from the fresh air duct were
405 calculated as 1.53 CFU s^{-1} and 0.94 CFU s^{-1} for bacteria and fungi, respectively.
406 After determining E_1, E_2 and E_3 from the previous scenarios, E_0 (the rate of supply of bioaerosols
407 from outdoors through the mechanical ventilation) became the only unknown parameter in Eq. (2)
408 for Scenario 4. Using a similar data fitting method based on Scenario 4's air sampling data (Fig.
409 S4, Supplemental Material), E_0 was calculated to be 1.64 CFU s^{-1} for bacteria and 8.48 CFU s^{-1} for
410 fungi.

411 Bioaerosol emanation rates E_0, E_1, E_2 and E_3, determined by experimental data fitting method
412 described above and the bioaerosol emanation rate due to resuspension from the floor are
413 summarized in Fig. 5. The result showed that the introduction of outdoor fungi through the
414 mechanical ventilation (E_0) served as the dominant source of indoor airborne fungi. The second
415 largest contributor to indoor airborne fungi was the emanation from the AC unit (E_2) but the
416 emanation rate is smaller than E_0 by a large margin. The combined fungal emanation rate from the
417 sources related to the ACMV system (E_2 + E_3) was only about half of E_0. This echoed the
418 observation from air sampling data (Fig. 4 comparison between Scenario 4 & 7) that using the
419 ACMV system was an effective measure to reduce indoor airborne fungal level, compared to
420 natural ventilation.
421 Emanation rates of bacteria from the four sources (E_0, E_1, E_2 and E_3) in the current experimental
422 setting were of similar magnitude. The combined bacterial emanation rate due to ACMV-related
423 sources (E_2 + E_3) out-weighted the emanation rate due to the introduction of outdoor bacteria
424 through the mechanical ventilation (E_0). It suggested that using the ACMV system could lead to
425 higher indoor airborne bacterial concentration compared to natural ventilation. This matches the
426 observation from air sampling data (Fig. 4, comparison between Scenario 4 & 7). The occupant
427 density in the current experimental setting was 18 m^2 $person^{-1}$, similar to the typical office
428 environment of 20 m^2 $person^{-1}$ (Kim and Haberl, 2012). However, for indoor environments with
429 higher occupant densities, e.g., classrooms (2.9 to 4 m^2 $person^{-1}$) or shopping malls (2.5 m^2 $person^{-1}$) (ASHRAE, 2004), bacteria emanation from occupants is expected to become the dominant
431 source for indoor airborne bacteria.
432

Species of Bioaerosol Emanated from the ACMV System

The species of bioaerosol samples collected from the fresh air duct (Figs. 3[a]) and those collected from the AC unit (Figs. 3[b]) were identified using the DNA sequencing method. The identified bacterial and fungal species are shown in Fig. 6 and Fig. 7 respectively. A number of 24 bioaerosol species from the fresh air duct and that of 39 bioaerosol species from the AC unit were identified. The number of bacterial species identified in the fresh air duct is close to that identified in the AC unit. In contrast, many more fungal species were identified in the AC unit than that identified in the fresh air duct, as shown in Fig. 7. A possible reason is that the fresh air duct only exposes to fresh air whereas the AC unit exposes to both the fresh air and the room return air. The room return air could contain more fungal species that are not in the fresh air, e.g., species shed from the occupants.

Some of the identified bacterial and fungal species are potential pathogenic species, as listed in the Updated Biological Agents and Toxins List (Ministry of Health Singapore, 2017). Among the identified bacterial species, three species in the *Staphylococcus* genera, *Staphylococcus cohnii sub.urealyticum*, which can cause catheters and urinary tract infection in immunocompromised patients (Shahandeh et al., 2015), *Staphylococcus warneri*, which might cause catheter-related infection (Kamath et al., 1992), and *Staphylococcus capitis*, which might cause prosthetic joint infection (Tevell et al., 2017), were found in the bioaerosol emanation from the ACMV system. A potential pathogenic bacterial species, *Moraxella osloensis*, which populate on the skin and mucosa of humans (Yamada et al., 2019), was only observed to be emanated from the AC unit, suggesting its transportation from occupants through room air recirculation and accumulation in the ACMV system.

All the potential pathogenic fungal species found in the ACMV system belong to the *Aspergillus* genera. *Aspergillus fumigatus*, which was found to be emanated from the fresh air duct, is a listed

potential pathogenic fungal species (Ministry of Health Singapore, 2017). Despite not being listed, the other two species from this genera, *Aspergillus sydowii* and *Aspergillus versicolor*, were also reported to be associated with several human diseases including the building-associated pulmonary diseases (Hodgson et al., 1998; Liu et al., 2017). The results of species identification indicate that potential pathogens, including those emanated from occupants, could accumulate in the ACMV system and subsequently being transported into the indoor space.

Mitigation of indoor bioaerosol pollution by ACMV cleaning

Results of the current study show that the ACMV system can be a significant source of indoor bioaerosols, especially bacteria. In order to investigate the potential mitigation of indoor bioaerosol pollution by better cleaning of the ACMV system, three hypothetical cases were simulated using Eq. (2):

1. Bioaerosol emanation of the fresh air duct was removed by setting $E_3 = 0$ (HYPO 1), simulating a cleaning of the fresh air duct. For bacteria, $E_0 = 1.64$ CFU s^{-1}, $E_1 = 1.11$ CFU s^{-1}, $E_2 = 0.87$ CFU s^{-1}. For fungi, $E_0 = 8.48$ CFU s^{-1}, $E_1 = 0.07$ CFU s^{-1}, $E_2 = 2.96$ CFU s^{-1}.
2. Bioaerosol emanation of the AC unit was removed by setting $E_2 = 0$ (HYPO 2), simulating a cleaning of the AC unit. For bacteria, $E_0 = 1.64$ CFU s^{-1}, $E_1 = 1.11$ CFU s^{-1}, $E_3 = 1.53$ CFU s^{-1}. For fungi, $E_0 = 8.48$ CFU s^{-1}, $E_1 = 0.07$ CFU s^{-1}, $E_3 = 0.94$ CFU s^{-1}.
3. Bioaerosol emanation of the ACMV system was removed by setting $E_2 = E_3 = 0$ (HYPO 3), simulating a cleaning of the entire ACMV system. For bacteria, $E_0 = 1.64$ CFU s^{-1}, $E_1 = 1.11$ CFU s^{-1}. For fungi, $E_0 = 8.48$ CFU s^{-1}, $E_1 = 0.07$ CFU s^{-1}.

The environmental parameters described before were used in this simulation. The AER and the bioaerosol emanation rates of sources in Scenario 4 were used as inputs for the simulation. These inputs are summarised in Table 1 and Table 2. The three hypothetical cases were compared to the

481 fitting of air sampling data obtained in Scenario 4 using Eq. (2), which was used as the reference
482 case in this comparison due to the fact that this scenario bore the closest resemblance to the actual
483 operating condition of the room under ACMV mode. The five-day average indoor bioaerosol
484 concentration at 9:30 am in Scenario 4 was used as the initial bioaerosol concentration C_0 in the
485 simulation (132.86 CFU m^{-3} for bacteria and 372.20 CFU m^{-3} for fungi). Simulation results are
486 shown in Fig. 8.

487 Compared to the reference case (Scenario 4), daily-integrated average indoor airborne bacterial
488 concentration drops by 28% in HYPO 1, 17% in HYPO 2 and 45% in HYPO 3. Similarly, daily-
489 integrated average indoor airborne fungal concentration drops by 8% in HYPO 1, 26% in HYPO 2
490 and 34% in HYPO 3. The results indicate that significant mitigation of indoor bioaerosol pollution
491 can be achieved if the hygiene of the ACMV system is improved. The air sampling results discussed
492 in earlier sections suggested that the ACMV mode leads to a higher indoor airborne bacterial
493 concentration compared to the natural ventilation mode. A major contributor to that is the bacterial
494 emanation from the ACMV system (E_2 and E_3). The HYPO 3 simulation results show that when
495 the bioaerosol emanation from the ACMV system is removed, the average indoor airborne bacterial
496 concentration will drop to about 50 CFU m^{-3}, which is around 30% less than the average indoor
497 airborne bacterial concentration under natural ventilation in Scenario 7 (about 72 CFU m^{-3}).

498

499 ***Limitations***

500 In this study, the culture-based method is employed, and this method is limited to the
501 investigation of the viable portion of bioaerosols. However, non-viable bioaerosols can also cause
502 health problems and the investigation of the non-viable portion of bioaerosol emanation from
503 ACMV systems is needed to have a comprehensive understanding of its role as a bioaerosol source.

Further study should be conducted to investigate the contamination and emanation of non-viable bioaerosols from ACMV systems.

The accuracy of the culture-based method is largely limited by colony formation. Since the impactor used has 200 holes, the maximum number of colonies countable on a sampling plate is 200. Too long a sampling time could cause overlapping of colonies on the sampling plate whereas too short a sampling time could lead to too few numbers of colonies on the sampling plate and thus high level of error (Sutton, 2011). This study used a sampling time of 10 minutes which gave colony counts ranging from 25 to 200 on most of the sampling plates, as recommended by Sutton (2011).

In the experimental room, the existence of bioaerosol sources might lead to a spatial inhomogeneity of indoor bioaerosol concentrations from Scenario 1 to 4, where the experimental data was used to quantify the contribution of bioaerosol sources using the indoor bioaerosol model with a well-mixed assumption (Eq. [1]). From Scenario 1 to 4, the experimental room had a relatively low air exchange rate (0.516-0.6 h^{-1}) combined with a relatively high air recirculation rate (around 13 h^{-1}), suggesting that the indoor air convection could rapidly and effectively mix the indoor air during the 7 hours' experiment in a day (Nazaroff and Cass, 1986) in these scenarios. In addition, bioaerosol concentration gradient due to deposition and resuspension only exits very close to the indoor surfaces (Lai and Nazaroff, 2000), indicating a reasonable hypothesis that the indoor bioaerosols were well mixed.

CONCLUSION

Bioaerosol emanation from an ACMV system and its impact on indoor bioaerosol pollution is investigated experimentally in an indoor environment in tropical Singapore. Surface and air sampling are conducted followed by culture-based analysis and species analysis. Bioaerosol species originated from both the outdoor and indoor sources, including the potential pathogenic

528 species emanated from occupants (*Moraxella osloensis*), can be found in the ACMV system. This
529 indicates the role of the ACMV system in indoor bioaerosol pollution as a potential pathogen source,
530 where bioaerosols can accumulate and be emanated. The dominating contributor to indoor airborne
531 fungi is the introduction of outdoor fungi through the mechanical ventilation, which is about double
532 of the contribution by the emanation from the ACMV system. This is backed by the fact that the
533 average indoor airborne fungal concentration measured during the ACMV mode is about half of
534 that measured during the natural ventilation mode. For bacteria, the emanation from the ACMV
535 system is the largest contributor to indoor airborne bacterial concentration compared to other
536 sources (the introduction of outdoor bacteria through the mechanical ventilation, the occupants'
537 emanation and the floor resuspension). The air sampling results suggest that operating the room in
538 ACMV mode results in a higher indoor airborne bacterial concentration than that in natural
539 ventilation mode (by 30%). Bacterial emanation from the occupants is a significant source, but
540 fungal emanation from the occupants is minimal. By cleaning the ACMV system, the indoor
541 airborne fungal and bacterial concentrations can be reduced by 34% and 45%, respectively.
542 Keeping the ACMV system clean can maintain both the indoor airborne fungal and bacterial
543 concentrations lower than those using natural ventilation.
544
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725 Table 1. Specific parameters used in indoor bioaerosol model.

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727 Table 2. Details of indoor bioaerosol sources in the experimental scenarios.

728

729 **Table 1.** Specific parameters used in indoor bioaerosol model.

	Bacteria		Fungi		Reference
	0.5 -1 μm	1 – 2.5 μm	2 - 3 μm	3 - 4 μm	
Indoor resuspension rate[a] (R_{floor}, 10^{-10} s^{-1})	1.22	50	50	50	(Zhou et al., 2011)
Indoor deposition velocity* (V_d, 10^{-5} m s^{-1})	1.67	9.72	9.72	36.1	(Zhou et al., 2011)
Filtration efficiency of the return air filter* (η_1)	0.04	0.09	0.12	0.12	(Burroughs, 2005)

[a]*Applicable range of aerodynamic diameter (μm) - Bacteria: 0.5 ~ 2.5 μm* (Wiedinmyer et al., 2004; Wiedinmyer et al., 2009)*, Fungi: 2 - 4 μm (Madsen et al., 2016)* .

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732 **Table 2.** Details of indoor bioaerosol sources in the experimental scenarios.

	Sources / sinks of indoor bioaerosols						
	AER	Introduction of outdoor bioaerosols through ventilation E_0	Number of human occupants E_1	AC unit		Fresh air duct E_3	Window
				Emanation E_2	Filtration by RAF η_1		
Scenario 1	0.516	No	0	Yes	Yes	No	Close
Scenario 2	0.516	No	2	Yes	Yes	No	Close
Scenario 3	0.588	No	2	Yes	Yes	Yes	Close
Scenario 4	0.69	Yes	2	Yes	Yes	Yes	Close
Scenario 5[a]	0.69	Yes	2	No	No	Yes	Close
Scenario 6[b]	0.69	Yes	2	Yes	No	Yes	Close
Scenario 7	1.63	Yes	2	No	No	No	Open

[a]The AC unit is switched OFF and disconnected from the fresh air duct
[b]The filter in the AC unit is taken off.
RAF – return air filter, AER – air exchange rate (hr^{-1}).
Bioaerosol resuspension from the floor occurs in all scenarios.

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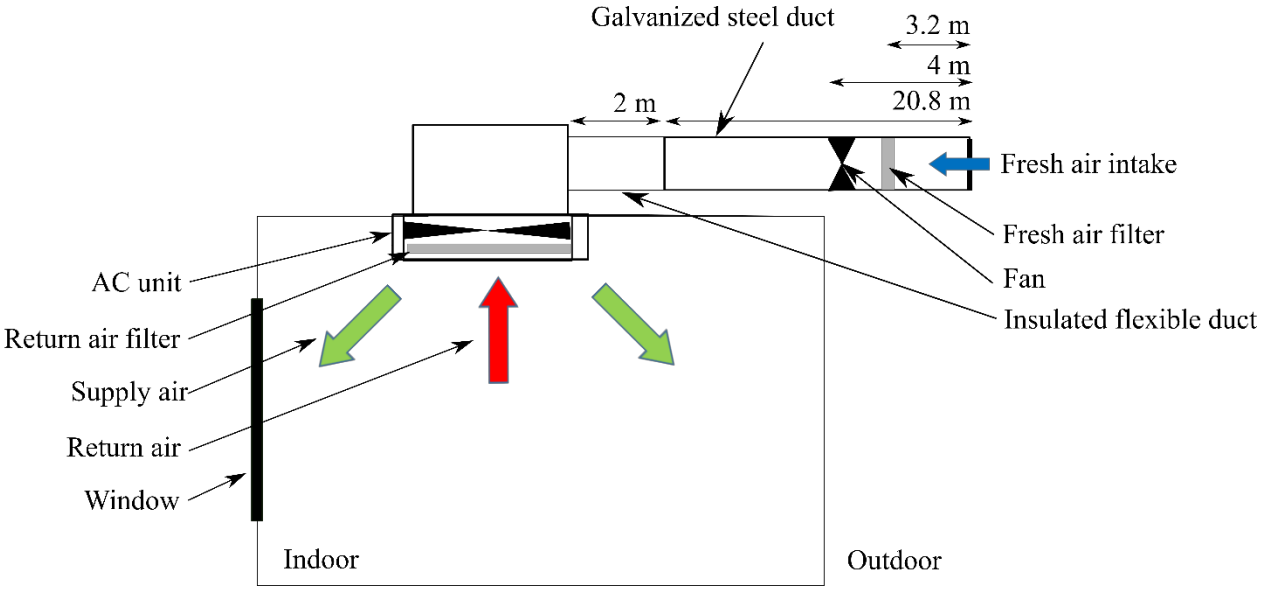

Fig. 1. Schematic diagram of the experimental tutorial room.

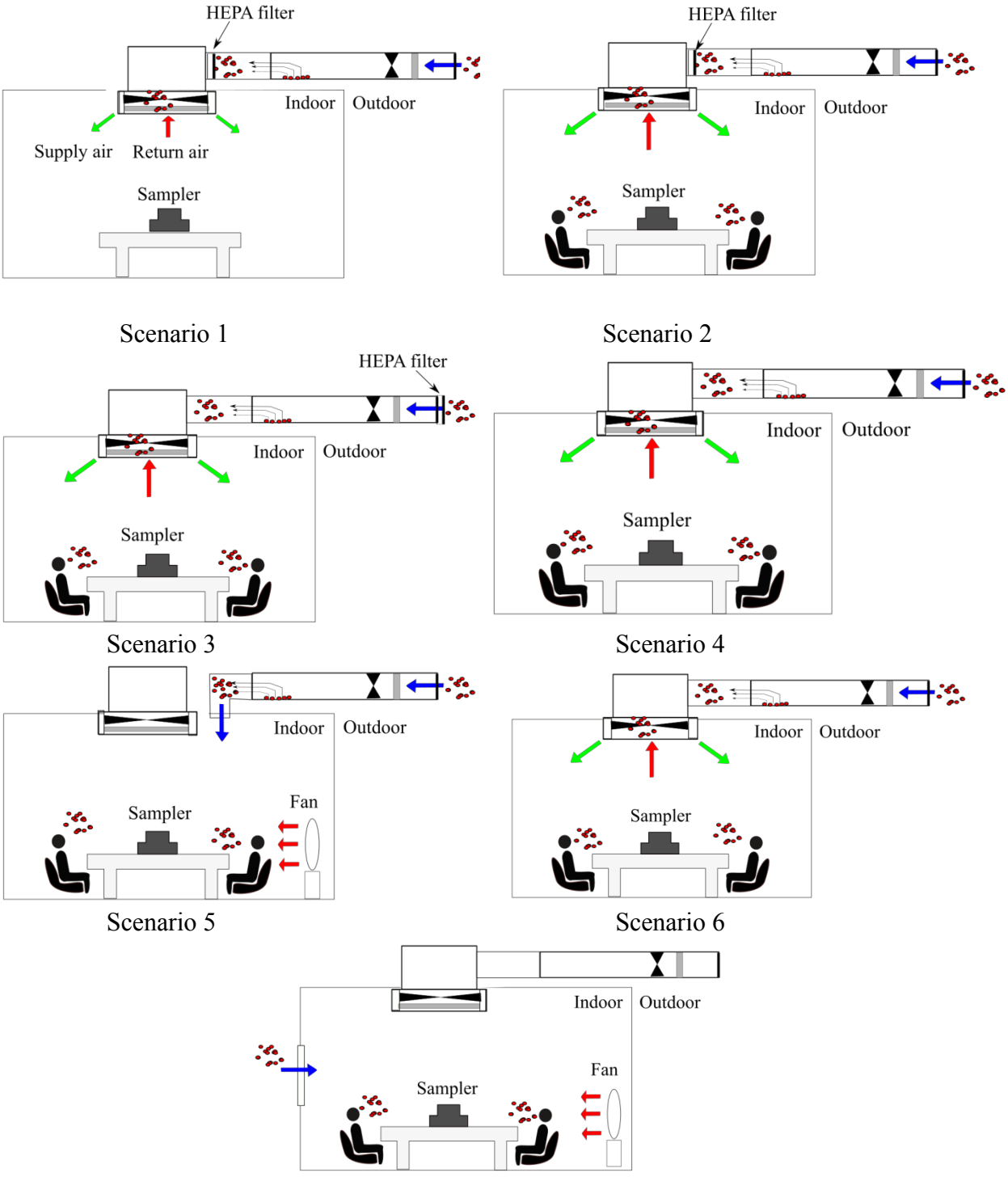

Fig. 2. Experimental setup for the seven scenarios.

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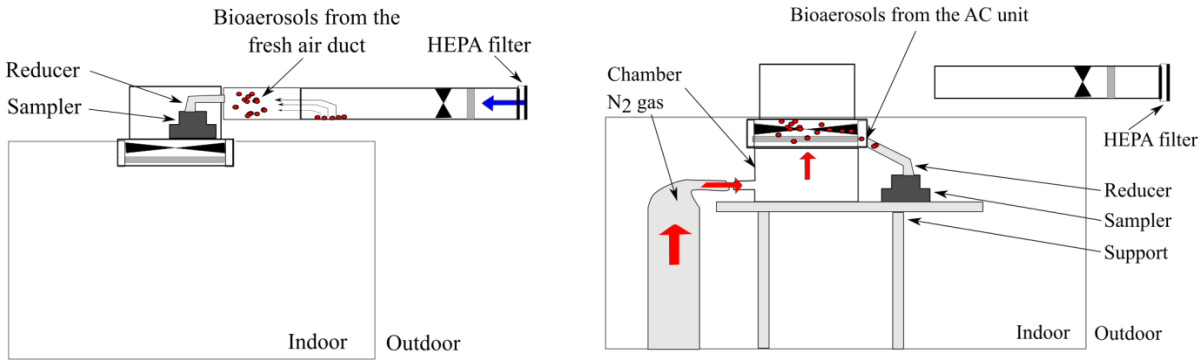

(a) Bioaerosol sampling in the fresh air duct (b) Bioaerosol sampling in the AC unit

781 **Figs. 3.** Experimental setup for species identification.

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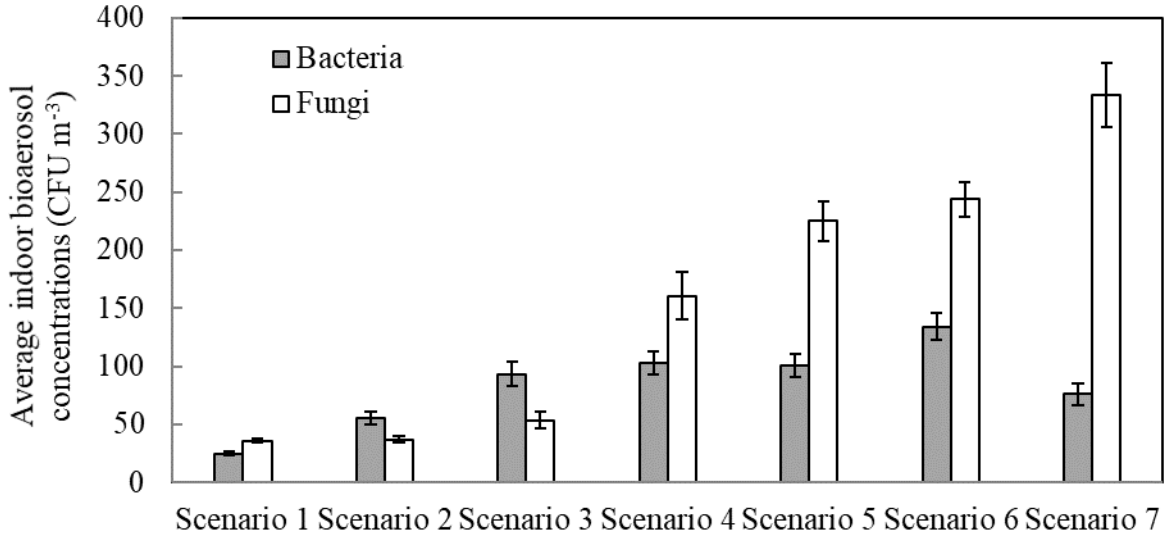

Fig. 4. Average indoor bioaerosol concentrations. Error bars show the standard error of mean from the five days' samples.

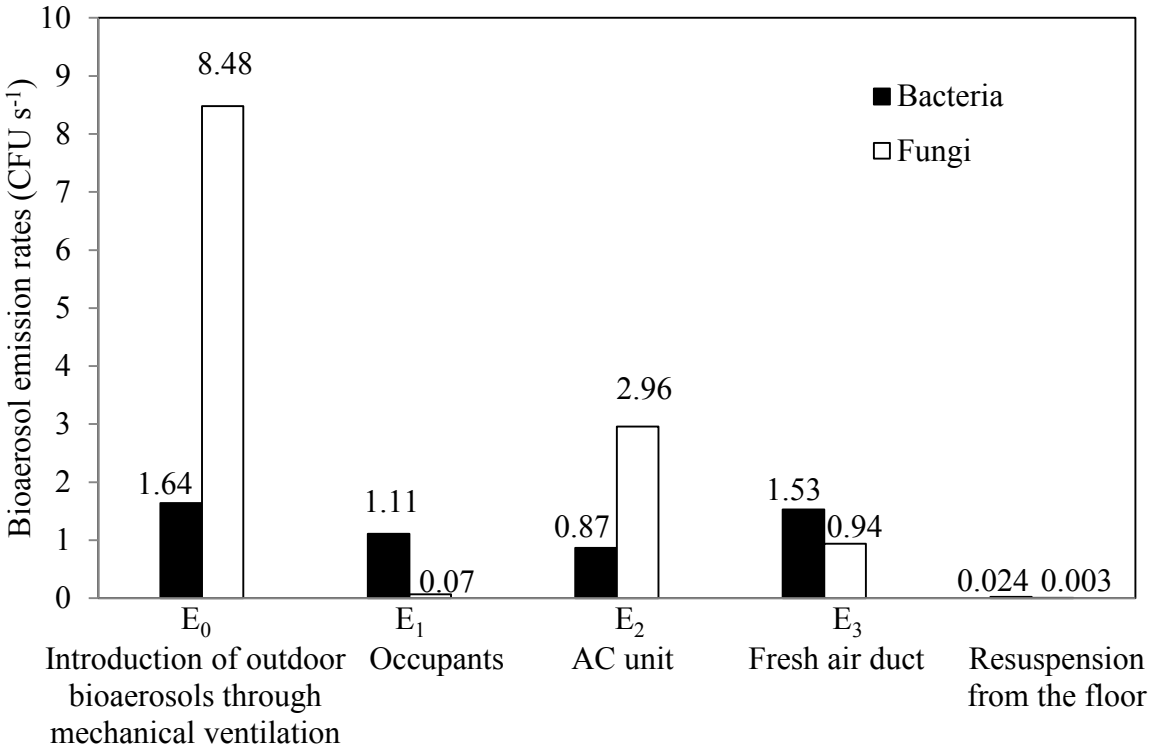

Fig. 5. Estimated bioaerosol emanation rates of bioaerosol sources.

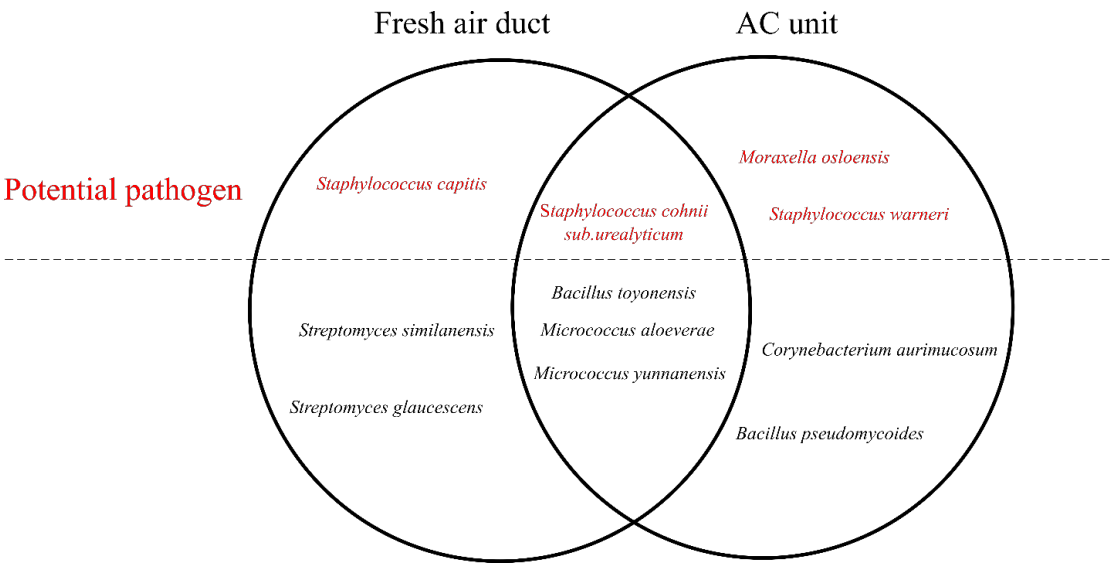

Fig. 6. Identified bacterial species emanated from the ACMV system.

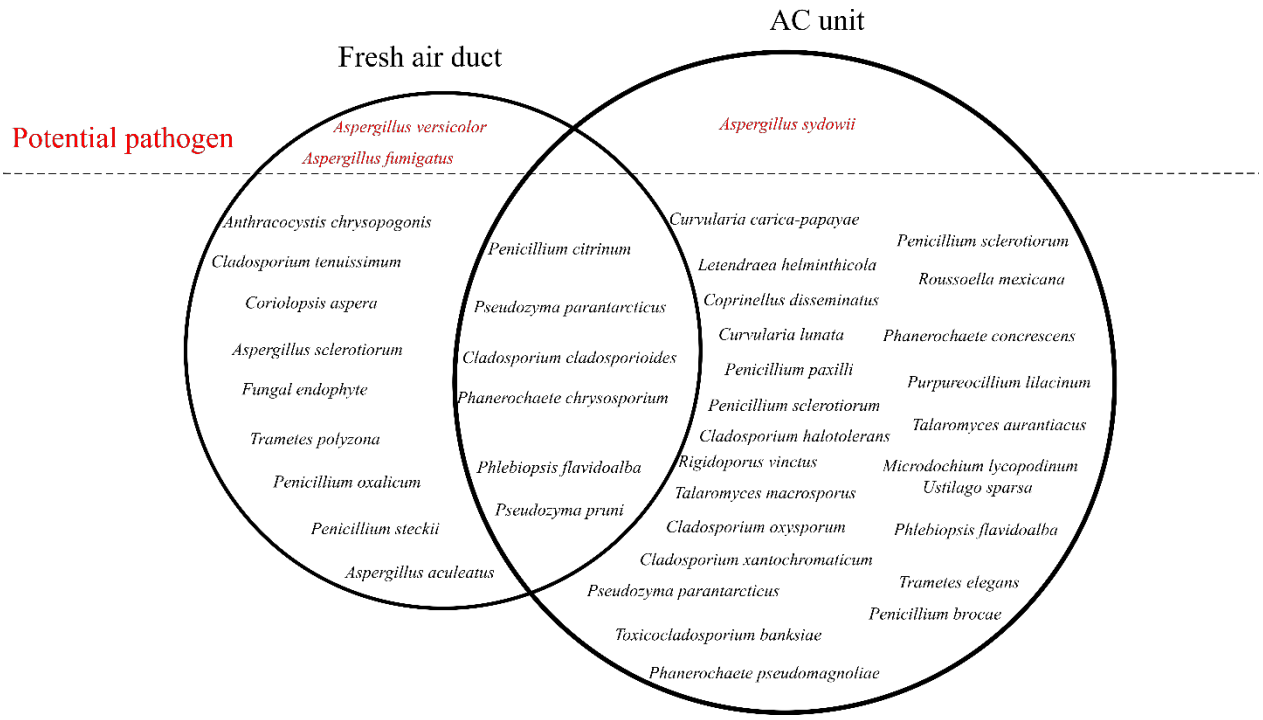

Fig. 7. Identified fungal species emanated from the ACMV system.

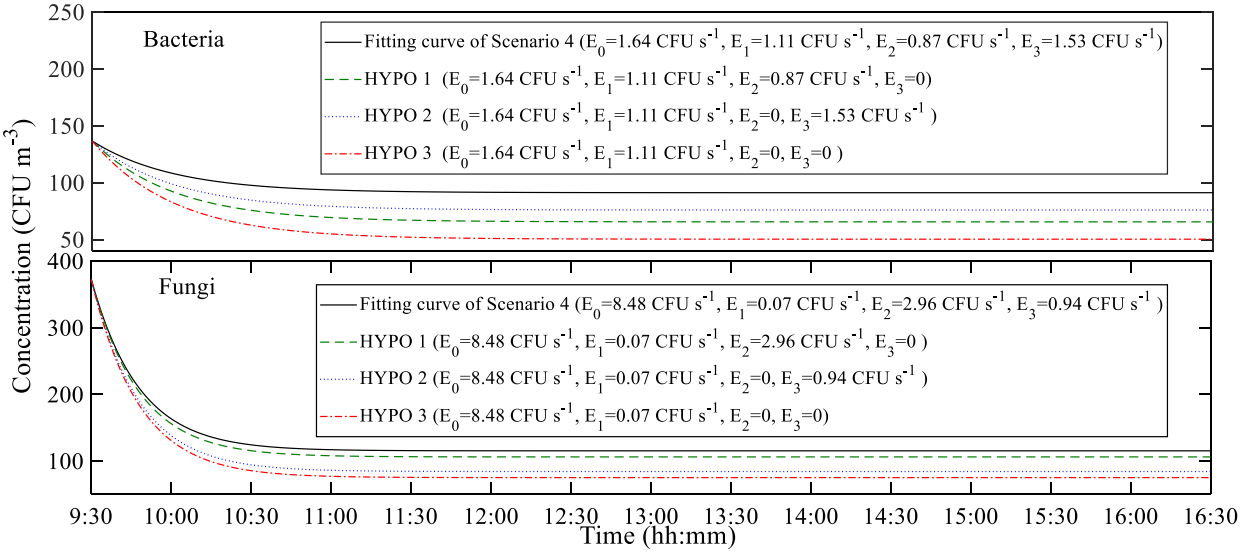

Fig. 8. Indoor bioaerosol concentrations (CFU m^{-3}) in the three hypothetical cases and the reference case (fitting of Scenario 4).